Statistical Analysis of Hormonal Effects on the Steroid Responsiveness of Solid Ehrlich Tumors

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SUMMARY

The influence of the gonads and adrenals on tumor growth was studied with SMA mice bearing solid Ehrlich tumors which have been produced by subcutaneous injection of ascitic Ehrlich tumor cells. It was found that the tumor growth was stimulated by the testes and inhibited by the ovaries and also by the administration of hydrocortisone acetate. The inhibitive effect of the adrenocortical hormone for tumor growth was observed only in male mice. The administration of testosterone and its two derivatives enhanced the tumor growth in castrated male and intact female mice, and estradiol suppressed it in intact male mice. A positive correlation for testosterone and a negative correlation for estradiol in the tumor response versus hormone dose were statistically significant. The diploid Ehrlich tumor was less responsive than the tetraploid Ehrlich tumor with respect to the effects of testosterone and hydrocortisone.

INTRODUCTION

The previous studies from our laboratory presented evidence that the development of Ehrlich ascites tumor is closely related to function of the adrenals and the gonads of the host. The castration experiment indicated that the growth of solid Ehrlich tumor was stimulated by the presence of the testes and inhibited by the presence of the ovaries (16, 17). In the hormone administration experiments however testosterone failed to give an appreciable effect on that tumor (17).

This study was initiated to investigate the action of various steroid hormones in greater detail, and thus to substantiate the hormone responsiveness of Ehrlich tumor.

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MATERIALS AND METHODS

All animals were SMA mice, propagated at the animal supply center of the Nagoya University School of Medicine by random mating. The hyperdiploid Ehrlich/2N ascites tumors and the hypotetraploid Ehrlich/4N ascites tumors were supplied in January 1963 by Dr. K. Kaziwara of the Takeda Research Laboratories, Osaka, Japan. These tumors were maintained in female mice by i.p. inoculation of 1 × 10^7 cells/mouse at 10-day intervals. Solid Ehrlich tumors were produced in mice by s.c. injection of ascitic Ehrlich tumor cells of the 8th inoculation day, 5 × 10^6/mouse, into the right shoulder region. The animals received a standard pellet diet (Oriental Yeast Mfg. Ltd., Tokyo, Japan), with drinking water ad libitum.

The following hormones were used: testosterone, Sigma Chemical Company, St. Louis, Mo.; 2-hydroxyethylene-17α-methyl dihydrotestosterone and 2α-methyl-3-oxo-5α-androstane-17β-ol-propionate, Syntex S. A., Mexico City, Mexico; estradiol, N. V. Organon; hydrocortisone acetate, Merck, Sharp and Dohme, West Point, Pa. With the exception of S-3900,1 the hormones were suspended in 0.9% NaCl solution before use. S-3900 was dissolved in sesame oil. They were all injected i.m. into the hind legs of the experimental mice. Control mice received the vehicles only. Castration was performed 1 month before tumor inoculation. Control animals underwent sham surgery. Data obtained were analyzed by Student's t test. For tumor weight, the deviation of the observed values from the normal distribution curve was estimated by the chi square test in an attempt to check the validity of the routine statistical procedures for an experiment, the data of which showed a great variability. Some of the statistical calculations were performed with a HITAC-201 computer.

RESULTS

The Deviation of the Distribution of Tumor Weight from the Normal Distribution. The early reports by Kodama (16, 17) showed that the variability of tumor weight was relatively large, and it was asked whether the distribution of

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1 The abbreviations used are: S-3900, 2α-methyl-3-oxo-5α-androstane-17β-ol-propionate; HCA, hydrocortisone acetate; HMD, 2-hydroxyethylene-17α-methyl dihydrotestosterone.
the data is sufficiently like a normal distribution to apply
the ordinary statistical analysis for this parameter. A total of
99 SMA male mice were inoculated s.c. with ascitic Ehrlich
tumor cells, and the animals were sacrificed 12 days later.
The weight of the excised tumors varied within the range of
151 to 6127 mg. The growth index of each tumor was
estimated by converting the weight (mg) into a common
logarithm which may represent the rate of cell proliferation
of the corresponding tumor providing that the cell division
proceeds geometrically. Table 1 shows the distribution of 99
tumor-bearing mice according to the tumor weight at right,
and also according to the logarithmic value of tumor weight
at left. The expected values are computed by determining
the proportion of the area of the normal distribution curve
at the corresponding class intervals. The chi square test was
applied for estimating the deviation of the above 2 sets of
distribution from the normal curve. The value of p for the
tumor weight is less than 0.0005, and the one for the
logarithm of tumor weight falls between 0.416 and 0.549.
These p values for the 2 sets of distribution were little
affected by a change in the magnitude of the classification
intervals. The conclusion is that the distribution of the
logarithm which may represent the rate of cell proliferation
proceeds geometrically. Table 1 shows the distribution of 99
mice with solid Ehrlich tumors according to tumor weight (mg) and also to the
logarithmic value of each tumor weight.

The Relationship between the Growth of Solid Ehrlich
Tumor and the Gonads. Ascitic tumor cells were inoculated s.c. on Day 0, and the animals were sacrificed on Day 15 for
tumor weighing. The mean tumor weight in castrated male mice
decreased progressively (Table 4). The body weight was also
increased parallel with the increase of hormone dose (Table
5). The hormone responsiveness of Ehrlich/2N and Ehrlich/4N
tumors was next compared in female mice. Testosterone, 0.2
mg and 1.0 mg/day/mouse, was administered on Days 1, 3,
5, 7, 9, 11, and 13. The tumors were weighed on Day 14. With the increase of hormone dose, the mean
tumor weight in male mice decreased progressively (Table 4).
There is no essential difference between Ehrlich/2N and
Ehrlich/4N tumors concerning the effect of estradiol.

Effect of Estradiol Administration on Tumor Growth. The
effect of male hormone was first investigated in castrated
male mice. Testosterone, 0.05 mg and 0.25 mg/day/mouse,
was administered on Days —11, —9, —7, —5, —3, —1, 1, 3,
5, 7, 9, and 11. Ehrlich/4N tumors were inoculated on Day
0. The animals were sacrificed on Day 14 for tumor weighing. The mean tumor weight in castrated male mice increased parallel with the increase of hormone dose (Table
5).

The hormone responsiveness of Ehrlich/2N and Ehrlich/4N
tumors was next compared in female mice. Testosterone, 0.2
mg and 1.0 mg/day/mouse, was administered on Days 1, 3,
5, 7, 9, 11, and 13. The tumors were weighed on Day 14.
Ehrlich/4N tumor in female mice was significantly stimulated
by the male hormone (Table 6). The body weight was also
increased by the same treatment, but no stimulative effect
was detected with Ehrlich/2N tumor in female mice. Thus
Ehrlich/2N tumor seems to be less hormone responsive than
Ehrlich/4N tumor with respect to HCA and testosterone.

In another experiment HMD, 0.5 mg and 2.0 mg/day/
mouse, and S-3900, 0.2 mg and 1.0 mg/day/mouse, were
administered on Days —11, —9, —7, —5, —3, —1, 1, 3,
5, 7, 9, 11, and 13. The tumors were weighed on Day 14.
Ehrlich/4N tumor was less responsive to HCA. Both tumors
were little affected in female mice by the same treatment.

Effect of HCA Administration on Tumor Growth. The
treatment with HCA, 1 mg/day/mouse, was started on Day 7
and ended on Day 13, a total of 7 injections. All animals
were killed on Day 14 and the solid tumors were excised
and weighed. HCA administration significantly suppressed
the growth of Ehrlich/4N tumor in male mice (Table 3).
Ehrlich/2N tumor was less responsive to HCA. Both tumors
were little affected in female mice by the same treatment.

<table>
<thead>
<tr>
<th>Range of tumor weighta</th>
<th>No. of mice observed/expectedb</th>
<th>Range of log10 (tumor weight, mg)a</th>
<th>No. of mice observed/expectedb</th>
</tr>
</thead>
<tbody>
<tr>
<td>0—500</td>
<td>13/9.2</td>
<td>2.1000—2.5000</td>
<td>4/3.0</td>
</tr>
<tr>
<td>501—1000</td>
<td>24/12.9</td>
<td>2.5000—2.7000</td>
<td>9/7.8</td>
</tr>
<tr>
<td>1001—1500</td>
<td>24/15.4</td>
<td>2.7000—2.9000</td>
<td>13/15.8</td>
</tr>
<tr>
<td>1501—2000</td>
<td>15/15.7</td>
<td>2.9000—3.1000</td>
<td>25/24.1</td>
</tr>
<tr>
<td>2001—2500</td>
<td>12/13.6</td>
<td>3.1000—3.3000</td>
<td>26/23.3</td>
</tr>
<tr>
<td>2501—3000</td>
<td>2/10.0</td>
<td>3.3000—3.5000</td>
<td>13/15.1</td>
</tr>
<tr>
<td>3001—3500</td>
<td>3/6.3</td>
<td>3.5000—3.7000</td>
<td>5/6.6</td>
</tr>
<tr>
<td>3501—4000</td>
<td>6/5.7</td>
<td>3.7000—4.1000</td>
<td>4/2.3</td>
</tr>
<tr>
<td>Total 99/88.8c</td>
<td>Total 99/99.0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

aThe mean values ± S.D. are 1554 ± 1235 for the set of 99 tumor weights and 3.0826 ± 0.3118 for
the logarithms of the weights of the same tumors.
bThe expected values are computed by determining the proportion of the area of the normal
distribution curve for the indicated class intervals.
cThe normal distribution curve for the given mean ± S.D. should have had a long tail on the negative
side of tumor weight so that the sum of the above 8 expected values on the positive side falls far
behind the observed value of 99.
Steroid Responsiveness of Solid Ehrlich Tumors

Table 2

Influence of the gonads for the growth of solid Ehrlich/4N and Ehrlich/2N tumors

<table>
<thead>
<tr>
<th>Group No.</th>
<th>Host</th>
<th>Gonadectomy</th>
<th>Mean body wt ± S.D. (g), based on (n) mice</th>
<th>Mean log_{10} (tumor wt, mg) ± S.D., based on (n) mice</th>
<th>p</th>
<th>p^a</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ehrlich/4N</td>
<td>Male</td>
<td>–</td>
<td>28.9 ± 2.1 (10)</td>
<td>2.8353 ± 0.4312 (10)</td>
<td>n.s.</td>
<td>0.02 &lt; p &lt; 0.05</td>
</tr>
<tr>
<td>1</td>
<td>Male</td>
<td>+</td>
<td>27.0 ± 2.0 (10)</td>
<td>2.3729 ± 0.4476 (10)</td>
<td>p &lt; 0.001</td>
<td>0.001 &lt; p &lt; 0.01</td>
</tr>
<tr>
<td>2</td>
<td>Female</td>
<td>–</td>
<td>24.9 ± 1.6 (10)</td>
<td>2.1922 ± 0.4212 (10)</td>
<td>0.001 &lt; p &lt; 0.01</td>
<td>0.001 &lt; p &lt; 0.01</td>
</tr>
<tr>
<td>1’</td>
<td>Female</td>
<td>+</td>
<td>21.5 ± 2.8 (9)</td>
<td>2.6841 ± 0.2869 (9)</td>
<td>n.s.</td>
<td>0.02 &lt; p &lt; 0.05</td>
</tr>
<tr>
<td>Ehrlich/2N</td>
<td>Male</td>
<td>–</td>
<td>33.7 ± 2.7 (19)</td>
<td>2.9943 ± 0.2538 (19)</td>
<td>p &lt; 0.001</td>
<td>0.02 &lt; p &lt; 0.05</td>
</tr>
<tr>
<td>1</td>
<td>Male</td>
<td>+</td>
<td>27.8 ± 2.7 (18)</td>
<td>2.7975 ± 0.2708 (18)</td>
<td>p &lt; 0.001</td>
<td>0.001 &lt; p &lt; 0.01</td>
</tr>
<tr>
<td>2</td>
<td>Female</td>
<td>–</td>
<td>24.5 ± 2.8 (20)</td>
<td>2.4331 ± 0.2438 (20)</td>
<td>n.s.</td>
<td>0.02 &lt; p &lt; 0.05</td>
</tr>
<tr>
<td>1’</td>
<td>Female</td>
<td>+</td>
<td>25.7 ± 2.2 (19)</td>
<td>2.4380 ± 0.2725 (19)</td>
<td>n.s.</td>
<td>0.02 &lt; p &lt; 0.05</td>
</tr>
</tbody>
</table>

p^a values are calculated from the logarithm of each tumor weight.

Numbers in parentheses represent no. of mice.

Not significant.

Table 3

Effect of hydrocortisone acetate on solid Ehrlich/4N and Ehrlich/2N tumors

<table>
<thead>
<tr>
<th>Group No.</th>
<th>Host</th>
<th>Daily dose of HCA (mg)</th>
<th>Mean body wt ± S.D. (g), based on (n) mice</th>
<th>Mean log_{10} (tumor wt, mg) ± S.D. based on (n) mice</th>
<th>p</th>
<th>p^a</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ehrlich/4N</td>
<td>Male</td>
<td>0</td>
<td>29.3 ± 1.9 (13)</td>
<td>2.8550 ± 0.2932 (13)</td>
<td>n.s.</td>
<td>p &lt; 0.001</td>
</tr>
<tr>
<td>1</td>
<td>Male</td>
<td>1.0</td>
<td>28.0 ± 1.5 (9)</td>
<td>2.2838 ± 0.2023 (9)</td>
<td>p &lt; 0.001</td>
<td>0.001 &lt; p &lt; 0.01</td>
</tr>
<tr>
<td>2</td>
<td>Female</td>
<td>0</td>
<td>22.5 ± 2.0 (15)</td>
<td>2.1074 ± 0.2020 (15)</td>
<td>n.s.</td>
<td>0.02 &lt; p &lt; 0.05</td>
</tr>
<tr>
<td>1’</td>
<td>Female</td>
<td>1.0</td>
<td>22.0 ± 2.2 (10)</td>
<td>2.1614 ± 0.1715 (10)</td>
<td>n.s.</td>
<td>0.02 &lt; p &lt; 0.05</td>
</tr>
<tr>
<td>Ehrlich/2N</td>
<td>Male</td>
<td>0</td>
<td>30.3 ± 1.1 (14)</td>
<td>2.7875 ± 0.1937 (14)</td>
<td>0.001 &lt; p &lt; 0.01</td>
<td>0.02 &lt; p &lt; 0.05</td>
</tr>
<tr>
<td>1</td>
<td>Male</td>
<td>1.0</td>
<td>26.8 ± 3.4 (9)</td>
<td>2.5834 ± 0.2487 (9)</td>
<td>p &lt; 0.001</td>
<td>0.001 &lt; p &lt; 0.01</td>
</tr>
<tr>
<td>2</td>
<td>Female</td>
<td>0</td>
<td>23.4 ± 2.5 (15)</td>
<td>2.3517 ± 0.3168 (15)</td>
<td>0.001 &lt; p &lt; 0.01</td>
<td>n.s.</td>
</tr>
<tr>
<td>1’</td>
<td>Female</td>
<td>1.0</td>
<td>20.4 ± 1.5 (9)</td>
<td>2.3147 ± 0.2156 (9)</td>
<td>n.s.</td>
<td>0.02 &lt; p &lt; 0.05</td>
</tr>
</tbody>
</table>

p^a values are calculated from the logarithm of each tumor weight.

Numbers in parentheses represent no. of mice.

Not significant.

given on Days -11, -9, -7, -5, -3, -1, 1, 3, 5, 7, 9, and 11. Ehrlich/4N tumor was inoculated on Day 0 and weighed on Day 14. As with testosterone, a significant stimulation of tumor growth was induced in female mice by these testosterone derivatives but not in male mice.

Correlation between the Hormone Dose and the Response from the Side of the Tumor and Host. Table 7 summarizes the correlation coefficients r and the p values for the response of the tumor and host versus the hormone doses which are calculated from the preceding data. There is a highly significant negative correlation between the dose of estradiol and the mean tumor weight in Ehrlich/2N and Ehrlich/4N tumors as well. A highly positive correlation was shown between the dose of testosterone and the mean weight of Ehrlich/4N tumors in the castrated male as well as in the intact female mice. However, this correlation was not significant for Ehrlich/2N tumor in female mice. The mean body weight and the mean uterine weight of female mice were also positively correlated to the dose of testosterone. The positive correlation between the dose of HMD and the mean weight of Ehrlich/4N tumor in female mice was statistically significant. Similarly, the correlation between the
Table 4

Effect of estradiol administration on solid Ehrlich/4N and Ehrlich/2N tumors

| Group No. | Host | Daily dose of estradiol (mg) | Mean body wt ± S.D. (g), based on (n) mice | Mean log10 (tumor wt, mg) ± S.D., based on (n) mice | p
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Ehrlich 4/N</td>
<td>1</td>
<td>Male 0</td>
<td>28.2 ± 2.6 (19)b</td>
<td>3.1336 ± 0.3918 (19)</td>
<td>p &lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>Male 0.2</td>
<td>26.0 ± 3.8 (19)</td>
<td>2.9410 ± 0.4218 (19)</td>
<td>n.s.</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>Male 1.0</td>
<td>28.9 ± 3.2 (20)</td>
<td>2.7318 ± 0.5012 (20)</td>
<td>0.001 &lt; p &lt; 0.01</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>Female 0</td>
<td>20.3 ± 2.5 (20)</td>
<td>2.4922 ± 0.3846 (20)</td>
<td>p &lt; 0.001</td>
</tr>
<tr>
<td>Ehrlich/2N</td>
<td>1</td>
<td>Male 0</td>
<td>23.4 ± 3.4 (21)</td>
<td>2.5508 ± 0.3730 (21)</td>
<td>n.s.</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>Male 0.2</td>
<td>23.6 ± 2.6 (10)</td>
<td>2.5557 ± 0.1422 (10)</td>
<td>n.s.</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>Male 1.0</td>
<td>21.7 ± 2.5 (10)</td>
<td>2.1834 ± 0.1767 (10)</td>
<td>0.001 &lt; p &lt; 0.01</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>Female 0</td>
<td>22.5 ± 1.2 (10)</td>
<td>2.2937 ± 0.3681 (10)</td>
<td>n.s.</td>
</tr>
</tbody>
</table>

aP values are calculated from the logarithm of each tumor weight.
bNumbers in parentheses represent no. of mice.
cNot significant.

Table 5

Effect of testosterone administration on solid Ehrlich/4N tumor in castrated male mice

| Group No. | Host | Gonadectomy | Daily dose of testosterone (mg) | Mean body wt ± S.D. (g), based on (n) mice | Mean log10 (tumor wt, mg) ± S.D., based on (n) mice | p
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Male</td>
<td>–</td>
<td>0</td>
<td>31.4 ± 1.6 (10)b</td>
<td>2.8978 ± 0.4515 (10)</td>
<td>p &lt; 0.001</td>
</tr>
<tr>
<td>2</td>
<td>Female</td>
<td>–</td>
<td>0</td>
<td>25.2 ± 1.9 (10)</td>
<td>1.9256 ± 0.4321 (10)</td>
<td>p &lt; 0.001</td>
</tr>
<tr>
<td>3</td>
<td>Male</td>
<td>+</td>
<td>0</td>
<td>28.4 ± 2.2 (10)</td>
<td>1.8404 ± 0.2429 (10)</td>
<td>p &lt; 0.001</td>
</tr>
<tr>
<td>4</td>
<td>Male</td>
<td>+</td>
<td>0.05</td>
<td>29.1 ± 1.7 (10)</td>
<td>2.2467 ± 0.3559 (10)</td>
<td>0.001 &lt; p &lt; 0.01</td>
</tr>
<tr>
<td>5</td>
<td>Male</td>
<td>+</td>
<td>0.25</td>
<td>32.9 ± 1.6 (11)</td>
<td>2.4435 ± 0.4849 (11)</td>
<td>0.001 &lt; p &lt; 0.01</td>
</tr>
</tbody>
</table>

aP values are calculated from the logarithm of each tumor weight.
bNumbers in parentheses represent no. of mice.
cNot significant.

dose of S-3900 and the mean weight of Ehrlich/4N tumor in female mice was significant at the 5% level.

DISCUSSION

Beatson's discovery in 1896 (1) of the suppressive effect of ovariectomy on human breast cancer was followed by many investigations on the experimental mammary cancer. Lacassagne (18) was the first to succeed in induction of breast cancer in male mice by weekly injections of 30 µg estrone benzoate, but, as Mühlbock (21) stated, most of the mammary tumors in mice with and without the tumor agent become hormone independent when they reached a palpable size.

The previous studies by Kodama (16) showed that the growth of solid Ehrlich tumor, a mammary cancer in origin, is closely related to the function of the gonads and the adrenals, but there remained some doubt as to the identification of hormones which are responsible for the stimulated growth of tumor in male mice (17). Also worth investigating is the significance of United States strains in the above experiments.

The present study with Japanese strains of mice and tumors reconfirmed the old findings about the suppressive effect of the corticosteroid hormone for the growth of solid Ehrlich tumor in male mice as well as the antagonistic relation between the male and female gonads in the same process (16, 17). Furthermore, the stimulative factor of the
Steroid Responsiveness of Solid Ehrlich Tumors

Table 6
Effect of testosterone administration on solid Ehrlich/4N and Ehrlich/2N tumors in intact female mice

<table>
<thead>
<tr>
<th>Group No.</th>
<th>Host</th>
<th>Daily dose of testosterone (mg)</th>
<th>Mean body wt ± S.D. (g), based on (n) mice</th>
<th>p</th>
<th>Mean log_{10} (tumor wt, mg) ± S.D., based on (n) mice</th>
<th>p^a</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ehrlich/4N</td>
<td>1</td>
<td>Female 0</td>
<td>20.6 ± 1.4 (20)</td>
<td>n.s.</td>
<td>2.5985 ± 0.3804 (20)</td>
<td>n.s.</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>Female 0.2</td>
<td>22.7 ± 2.9 (19)</td>
<td>p &lt; 0.001</td>
<td>2.8722 ± 0.4963 (19)</td>
<td>p &lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>Female 1.0</td>
<td>24.2 ± 1.4 (19)</td>
<td>p &lt; 0.001</td>
<td>3.1155 ± 0.2617 (19)</td>
<td>p &lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>Male 0</td>
<td>28.7 ± 2.5 (20)</td>
<td>p &lt; 0.001</td>
<td>3.2133 ± 0.2784 (20)</td>
<td>p &lt; 0.001</td>
</tr>
<tr>
<td>Ehrlich/2N</td>
<td>1</td>
<td>Female 0</td>
<td>21.8 ± 1.5 (20)</td>
<td>0.01 &lt; p &lt; 0.02</td>
<td>2.7343 ± 0.3021 (20)</td>
<td>n.s.</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>Female 0.2</td>
<td>22.6 ± 1.6 (19)</td>
<td>0.01 &lt; p &lt; 0.02</td>
<td>2.3150 ± 0.3388 (19)</td>
<td>n.s.</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>Female 1.0</td>
<td>23.3 ± 1.5 (20)</td>
<td>p &lt; 0.001</td>
<td>2.4345 ± 0.3487 (20)</td>
<td>p &lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>Male 0</td>
<td>27.5 ± 3.0 (20)</td>
<td></td>
<td>2.9967 ± 0.2877 (20)</td>
<td>p &lt; 0.001</td>
</tr>
</tbody>
</table>

*p values are calculated from the logarithm of each tumor weight.
Numbers in parentheses indicate no. of mice.
Not significant.

Table 7
Correlation between the hormone dose (x) and the response (y) of the tumor and host

<table>
<thead>
<tr>
<th>x</th>
<th>y</th>
<th>Host</th>
<th>r^a</th>
<th>p^a</th>
</tr>
</thead>
<tbody>
<tr>
<td>Estradiol (mg/day/mouse)</td>
<td>Mean weight of Ehrlich/4N tumor (mg)</td>
<td>Male mice</td>
<td>-0.34</td>
<td>0.009 &lt; p &lt; 0.013</td>
</tr>
<tr>
<td>Testosterone (mg/day/mouse)</td>
<td>Mean body weight (g)</td>
<td>Castrated male mice</td>
<td>0.25</td>
<td>n.s.</td>
</tr>
<tr>
<td></td>
<td>Mean weight of Ehrlich/4N tumor (mg)</td>
<td>Castrated male mice</td>
<td>0.50</td>
<td>0.005 &lt; p &lt; 0.007</td>
</tr>
<tr>
<td></td>
<td>Mean weight of Ehrlich/4N tumor (mg)</td>
<td>Female mice</td>
<td>0.48</td>
<td>0.002 &lt; p &lt; 0.004</td>
</tr>
<tr>
<td></td>
<td>Mean body weight (g)</td>
<td>Female mice</td>
<td>0.44</td>
<td>p = 0.001</td>
</tr>
<tr>
<td></td>
<td>Mean uterine weight (mg)</td>
<td>Female mice</td>
<td>0.29</td>
<td>0.027 &lt; p &lt; 0.036</td>
</tr>
<tr>
<td>HMD (mg/day/mouse)</td>
<td>Mean weight of Ehrlich/4N tumor (mg)</td>
<td>Male mice</td>
<td>0.11</td>
<td>n.s.</td>
</tr>
<tr>
<td>S-3900 (mg/day/mouse)</td>
<td>Mean weight of Ehrlich/4N tumor (mg)</td>
<td>Female mice</td>
<td>0.39</td>
<td>0.021 &lt; p &lt; 0.028</td>
</tr>
</tbody>
</table>

For tumor tissue, the r and p values are calculated from the logarithm of each tumor weight.
Not significant.
testes for the tumor growth was identified as testosterone through the hormone administration experiment. The total body weight showed a relatively small deviation, whereas a great fluctuation was observed in the weight of neoplastic tissue which is beyond the homeostatic control of the host. Experimental data with a greater standard deviation may render some difficulty in applying the t test for statistical evaluation. The logarithm of each measurement could be sometimes more appropriate for that purpose, as suggested by Bross (3). In our case, the logarithm of tumor weight may serve as a growth index of the corresponding tumor provided that the tumor growth proceeds as an exponential function of inoculation day. Our old findings on the growth of solid Ehrlich tumor showed a very slight deviation from the exponential increase (17). The chi square test was applied for these data to see whether or not they are distributed normally enough to permit the use of the mean, standard deviation, and table of areas of the normal curve in describing a set of measurements. It is clear that the distribution of logarithmic values of tumor weight is essentially normal, making a sharp contrast to the distribution of tumor weight itself which shows a remarkable deviation from the normal curve. By the same test the distributions of the weight of the liver, the kidney, and the total body were normal.

There are several reports that the male hormone may stimulate the growth of mammary cancer (5, 10, 25, 26). Mineshita and Yamaguchi (20) reported on an androgen-dependent mammary tumor of mouse which failed to grow in the absence of the male hormone. Furth (8) also stated that a “male type” of mammary tumor first induced in a male rat preferred the male host in successive transplantation. These findings indicate that the hormone dependency of cancer cells can be produced under the influence of hormonal milieu intérieur. The stimulative effect of testosterone in this case was observed with the Ehrlich tumors which had been maintained in female mice over 3 years.

The hypotetraploid tumor seems to be more responsive than the hyperdiploid tumor with regard to the effect of testosterone and hydrocortisone. The same difference between the above 2 tumors has also been noted in their susceptibility to 6-mercaptopurine (22). Some discrepancies between our data and those of others (9, 24) might be explained by the difference in the tumors or in the experimental procedures. The inhibitive effect of hydrocortisone is observed only in male mice bearing solid Ehrlich/4N tumors. The most probable explanation for our findings is that the growth of solid Ehrlich/4N tumor consists of 2 portions; one portion is steroid insensitive and the other is steroid sensitive. In female mice the growth of solid Ehrlich tumor proceeds only through the former process in the absence of stimulative action of testosterone. In male mice the growth of solid Ehrlich tumor is a summation of the first and the second processes, the latter being stimulated by the male hormone of the host. If hydrocortisone is administered to male mice, the second portion of tumor growth is extinguished by an inhibitive action of the hormone. In female mice no inhibition is observed in the tumor growth, which is entirely steroid independent. There is no information available as to whether the presence of steroid-sensitive and -insensitive growth is related to the inhomogeneity of the cell population of the Ehrlich tumor.

The protein-anabolic action of testosterone was noted by Kochakian et al. (14, 15) who found that testosterone increased the carcass weight of the rodents with no significant change in the proportion of protein, fat, or water. In the radioisotope experiment, the incorporation of labeled amino acids into proteins of various organs was increased by the pretreatment with testosterone (4, 6, 7). Evidence indicates that the most probable action of testosterone is to influence the concentration of template (messenger) RNA of ribosomes (19, 27).

Kim et al. (11) emphasized the significance of mammotropic hormone in the growth of estrogen-responsive mammary tumor; they showed that estradiol failed to stimulate the growth of mammary tumor in hypophysectomized rats, while mammotropin was highly effective in stimulating tumor growth. It was concluded that the stimulatory action of estradiol for mammary tumor results from stimulation of pituitary mammotropes. For Ehrlich tumor, the possible participation of the pituitary in the steroid hormone assays is not excluded, but there are indications that some of the androgen actions are independent of the pituitary (12, 13, 15, 23).

Blum (2) stated that the growth of cancer might be either an expression of the overall growth of the animal or a function of the specific factor which governs only the growth of the target tissue. The action of hormones as measured in terms of tumor weight was not specifically differentiated in this respect. The present investigation indicated a stimulative effect of testosterone and an inhibitive effect of hydrocortisone and estradiol for the growth of solid Ehrlich tumor. There is a possibility that the administration of a hormone will affect the food intake of the host animal which may in turn influence the tumor growth. The significance of host factors which might be involved in the expression of hormonal action is to be elucidated in future. Some of the metabolic changes in hormone-treated tumor cells will be described in a subsequent publication.

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Steroid Responsiveness of Solid Ehrlich Tumors


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Mitsuo Kodama and Toshiko Kodama


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